



**HAL**  
open science

## Population dynamics of tuberculous Bacilli in Cameroon as assessed by spoligotyping.

Francioli Koro Koro, Yannick Kamdem Simo, Félix Fotso Piam, Jurgen Noeske, Cristina Gutierrez, Christopher Kuaban, Sara Irène Eyangoh

### ► To cite this version:

Francioli Koro Koro, Yannick Kamdem Simo, Félix Fotso Piam, Jurgen Noeske, Cristina Gutierrez, et al.. Population dynamics of tuberculous Bacilli in Cameroon as assessed by spoligotyping.. *Journal of Clinical Microbiology*, 2013, 51 (1), pp.299-302. 10.1128/JCM.01196-12 . pasteur-00835858

**HAL Id: pasteur-00835858**

**<https://riip.hal.science/pasteur-00835858>**

Submitted on 20 Jun 2013

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

## Population Dynamics of Tuberculous Bacilli in Cameroon as Assessed by Spoligotyping

Francioli Koro Koro, Yannick Kamdem Simo, Félix Fotso Piam, Jurgen Noeske, Cristina Gutierrez, Christopher Kuaban and Sara Irène Eyangoh

*J. Clin. Microbiol.* 2013, 51(1):299. DOI:  
10.1128/JCM.01196-12.

Published Ahead of Print 31 October 2012.

---

Updated information and services can be found at:  
<http://jcm.asm.org/content/51/1/299>

---

*These include:*

**REFERENCES**

This article cites 17 articles, 7 of which can be accessed free at:  
<http://jcm.asm.org/content/51/1/299#ref-list-1>

**CONTENT ALERTS**

Receive: RSS Feeds, eTOCs, free email alerts (when new articles cite this article), [more»](#)

---

---

Information about commercial reprint orders: <http://journals.asm.org/site/misc/reprints.xhtml>  
To subscribe to to another ASM Journal go to: <http://journals.asm.org/site/subscriptions/>

---

# Population Dynamics of Tuberculous Bacilli in Cameroon as Assessed by Spoligotyping

Francioli Koro Koro,<sup>a,b</sup> Yannick Kamdem Simo,<sup>a</sup> Félix Fotso Piam,<sup>a</sup> Jurgen Noeske,<sup>c</sup> Cristina Gutierrez,<sup>d</sup> Christopher Kuaban,<sup>e</sup> Sara Irène Eyangoh<sup>a</sup>

Mycobacteriology Service, Reference Laboratory of NTP, Centre Pasteur of Cameroon, Cameroon-Pasteur Institute International Network, Yaoundé, Cameroon<sup>a</sup>; University of Yaoundé I, Faculty of Sciences, Department of Biochemistry, Yaoundé, Cameroon<sup>b</sup>; German International Cooperation (GIZ), Yaoundé, Cameroon<sup>c</sup>; Foundation for Innovative New Diagnostics, Geneva, Switzerland<sup>d</sup>; University of Yaoundé I, Faculty of Medicine and Biomedical Sciences/Jamot Hospital, Yaoundé, Cameroon<sup>e</sup>

**Genetic assessment by spoligotyping of 565 *Mycobacterium tuberculosis* complex strains collected from the Western Region of Cameroon between 2004 and 2005 has confirmed the establishment of the “Cameroon family” as the leading cause of tuberculosis in 45.9% of cases and evidenced the rapid quasi extinction of *Mycobacterium africanum*, isolated in 3.3% of tuberculosis cases.**

Tuberculosis (TB) remains a major cause of illness and death worldwide, especially in Africa and Asia (1). In Cameroon, the incidence of all clinical forms of tuberculosis is about 25,000 new cases per year (National Tuberculosis Programme [NTP] report no. 16, May 2011).

The understanding of tuberculosis transmission dynamics and epidemiology has been greatly enhanced by molecular epidemiologic studies using various DNA typing techniques in conjunction with classical epidemiological approaches (2). These techniques exploit various DNA elements as markers to assess the overall dissemination of strains. One such method is spoligotyping, which analyzes DNA polymorphism observed in the spacer sequences found in the direct repeat (DR) locus of the *Mycobacterium tuberculosis* complex (MTBC) genome. This technique is simple, rapid, and robust and has the advantage of enabling simultaneous distinction between MTBC isolates at subspecies level. It also measures overall diversity, thus providing information about major lineages. Furthermore, an international database has been established for the purpose of comparison of MTBC spoligotypes (3).

An initial retrospective study on the characterization of *M. tuberculosis* genotype strains collected in the Western Region of Cameroon was carried out between July 1997 and June 1998. This area was chosen by the NTP as a model to assess the impact of various interventions. The application of spoligotyping showed the striking regression of *Mycobacterium africanum* as an etiologic agent of tuberculosis from 50% in the 1970s to 9% in the 1990s (4). This study also showed the predominance of a group of strains named “Cameroon family” strains, representing 43% of tuberculosis cases and designated LAM-10 CAM in SpolDB4. Further analysis confirmed that the lack of spacers 22, 23, and 24 was the specific signature of this family due to the removal of IS6110 in the DR region (5).

To further investigate the genetic diversity evolution and the dynamics of the dissemination of strains in the Western Region of Cameroon, spoligotyping was used to perform a retrospective analysis on a new collection of strains obtained during a 1-year survey (February 2004 to March 2005) to evaluate the impact of tuberculosis control on resistance to antituberculosis drugs, 7 years after the first study.

**Bacterial strains and spoligotyping.** Bacterial strains used in

this study were isolated at the Mycobacteriology Reference Laboratory of the Centre Pasteur of Cameroon from pulmonary tuberculosis patients in the Western Region over a 1-year period (February 2004 to March 2005). All sputum smear-positive cases aged more than 15 years were included in the prospective survey. One sputum sample was collected from each patient in a transport medium solution (0.6% cetyl pyridinium bromide) and sent to the Mycobacteriology Reference Laboratory. Each sample was cultured on Löwenstein-Jensen (LJ) medium and LJ medium supplemented with 0.4% pyruvate. We isolated 622 MTBC strains identified by biochemical and phenotypical methods: 25 of *M. africanum*, 596 of *M. tuberculosis*, and one of *Mycobacterium bovis*. Of the 622 strains of MTBC isolated and kept frozen, 565 MTBC isolates were successfully subcultured on LJ medium and LJ medium supplemented with 0.4% pyruvate. The rest of the isolates could not be typed due to either contamination or the inability to revive them from subculture. DNA was extracted from each strain by using colonies grown on LJ medium. Spoligotyping to detect the 43 spacers was performed at the Mycobacteriology Reference Laboratory of the Centre Pasteur Cameroon using a commercially available kit (Isogen Biosciences BV, Maarsse, the Netherlands) as previously described (6). Results were entered into Microsoft Excel and compared with an international database, SITVIT2. Spoligotypes which could not be matched with those in the database were considered novel. For the other strains, the spoligotyping defined shared international types (SITs), and the corresponding family was designated. The Bionumerics program, version 5.10 (Applied Maths, Kortrijk, Belgium), was also used for comparison and dendrogram construction.

**Biodiversity analysis.** A total of 94 distinct spoligotypes were obtained from the set of 565 MTBC strains. Of the 94 spoligotypes,

Received 13 May 2012 Returned for modification 14 June 2012

Accepted 22 October 2012

Published ahead of print 31 October 2012

Address correspondence to Sara Irène Eyangoh, eyangoh@pasteur-yaounde.org.

Copyright © 2013, American Society for Microbiology. All Rights Reserved.

doi:10.1128/JCM.01196-12



FIG 1 Dendrogram and schematic representation of 94 spoligotypes identified from 565 *M. tuberculosis* complex strains isolated from positive pulmonary tuberculosis patients from West Cameroon. The degree of similarity of spoligotypes was calculated with the 1-Jaccard index, and the relationships between patterns were assessed by the unweighted-pair group method using average linkages. The spoligotype code, the shared international type (SIT), and lineage are listed. ND, not determined. CAM, Cameroonian spoligotype corresponding to a strain isolated in this study.

49 patterns matched those found in SpolDB4 and 45 were identified as novel (Fig. 1).

Of the total 565 MTBC strains, 19 were identified as *M. africanum*, representing 3.3% of MTBC strains. This result shows a real regression of *M. africanum* compared to the 9% described in the 1990s ( $P = 0.0001$ ) (4). *M. africanum* appears to be really polymorphic; the 19 strains were divided into 13 spoligotypes, 7 of which were novel. The Afri-2 family previously reported as a majority in West African countries counted 15 strains (7).

The 546 strains identified as *M. tuberculosis* were split into 81 different spoligotypes. Of the 565 MTBC strains, a total of 260 strains belonged to the Cameroon family, representing 45.9% and 47.6%, respectively, of the *M. tuberculosis* isolates, compared with 43% and 47%, respectively, found in the late 1990s (4). The largest cluster of this family was formed by 186 isolates grouped in SIT 61. The other commonly described lineages were Haarlem (103 isolates), T1 (55 isolates), and T2 (48 isolates), which together showed an increasing trend. Figure 2 shows the evolution of the

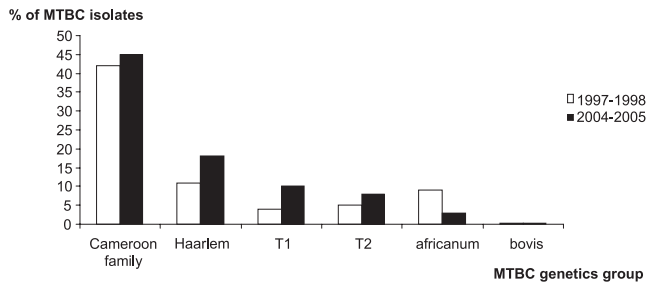


FIG 2 Histogram showing the 7-year evolution of the MTBC population in West Cameroon.

MTBC population over a period of 7 years. As was the case in our previous study (4), none of the strains presented the spoligotype of the worldwide-expanded epidemic Beijing family; one strain was identified as *M. bovis*.

Analysis of genetic diversity of strains in the Western Region of Cameroon shows that the Cameroon family is well established and comprises nearly half of the isolates. This family belongs to the modern lineage group 2, including major epidemic strains (8). The Cameroon family, as suggested by different studies on the Beijing family (9), could also have some selective advantages over other *M. tuberculosis* genotypes present in Cameroon, including virulence, pathogenesis, and epidemiologic characteristics. However, geographic confinement could also explain the predominance of the Cameroon family. Data on the genetic diversity of MTBC in sub-Saharan Africa suggested that this family is mainly found in Central and West African and Caribbean countries (5). Other, larger studies should be carried out for bordering countries.

The analysis also shows that *M. africanum* seems to be disappearing in Cameroon, a Central African country, unlike in West African countries, which continue to report between 20% and 39% of cases attributable to this species (7, 10–13). Previous studies have raised potential limitations concerning the difficulties in isolating and identifying *M. africanum* due to metabolic constraints (14) and the bias that may have characterized studies that concluded that *M. africanum* was regressing in Africa (11). In our laboratory, we avoid these limitations by using for primoculture LJ medium supplemented with 0.4% pyruvate, the culture medium that has to date shown a better recovery rate for *M. africanum* than that in other media. Other studies using MGIT Bactec medium for primoculture found higher rates of *M. africanum* (15, 16). We have recently processed 1,920 respiratory specimens (from June 2011 to June 2012) using in parallel LJ medium, LJ-pyruvate medium, and the MGIT Bactec medium for primoculture, and we observed that the addition of MGIT Bactec has not increased the recovery of *M. africanum* in our setting (unpublished data; personal observation). These data are in favor of a real difference from West African countries and support the geographical pattern of *M. africanum* distribution found by de Jong et al. (7). Unless the recovery of *M. africanum* is difficult due to well-known metabolic constraints which could bias its prevalence, the noted regression in Cameroon seems genuine, since the results of 2 studies performed under the same conditions and in the same area were compared. In Cameroon, despite the high polymorphism of *M. africanum*, the mean age of *M. africanum*-infected people is 33 years (range, 19 to 69 years), and this is an indication

of an ongoing transmission. Larger studies should be carried out under optimal conditions for *M. africanum* recovery in order to better define its distribution in terms of the geographical location of the country.

The dynamic approach of tracing changes in the MTBC population structure over years is not commonly applied, but it could contribute to highlighting clade-specific pathogenic characteristics (17).

Our study provides the first depiction of the molecular population dynamics of *M. tuberculosis* complex strains in Cameroon over a 7-year period. The reasons for the successful adaptation of the Cameroon family, the regression of *M. africanum*, and the absence of Beijing strains need to be explored in order to better understand the major forces driving the transmission dynamics within specific populations, which may significantly impact TB control and vaccine development strategies.

#### ACKNOWLEDGMENTS

Francioli Koro Koro is a Ph.D. student of the University of Yaoundé I. This project did not receive external grant funding. The cost for genotyping was covered by the Centre Pasteur of Cameroon's internal resources. We acknowledge the support of the Centre Pasteur of Cameroon in the realization of this research.

S.I.E. and C.K. conceived and designed the experiments. S.I.E., F.K.K., Y.K.S., and F.F.P. performed the experiments. S.I.E., F.K.K., and C.G. analyzed the data. S.I.E. wrote the first draft of the paper. C.G. designed figures. All authors provided critical input.

#### REFERENCES

- Jassal MS, Bishai WR. 2010. Epidemiology and challenges to the elimination of global tuberculosis. *Clin. Infect. Dis.* 50:S156–S164.
- van der Spuy G, Warren R, van Helden P. 2009. The role of molecular epidemiology in low-income, high-burden countries. *Int. J. Tuberc. Lung Dis.* 13:419–420.
- Brudey K, Driscoll J, Rigouts L, Prodinger W, Gori A, Al-Hajjaj S, Allix C, Aristimuno L, Arora J, Baumanis V, Binder L, Cafrune P, Cataldi A, Cheong S, Diel R, Ellermeier C, Evans J, Fauville-Dufaux M, Ferdinand S, Garcia de Viedma D, Garzelli C, Gazzola L, Gomes H, Guttierrez M, Hawkey P, van Helden P, Kadiwal G, Kreiswirth B, Kremer K, Kubin M, Kulkarni S, Liens B, Lillebaek T, Ho M, Martin C, Mokrousov I, Narvskaia O, Ngeow Y, Naumann L, Niemann S, Parwati I, Rahim Z, Rasolofon-Razanamp V, Rasolonavalona T, Rossetti M, Rusch-Gerdes S, Sajduda A, Samper S, Shemyakin I, Singh U, Somoskov A, Skuce R, van Soolingen D, Streicher E, Suffys P, Tortoli E, Tracevska T, Vincent V, Victor T, Warren R, Yap S, Zaman K, Portaels F, Rastogi N, Sola C. 2006. Mycobacterium tuberculosis complex genetic diversity: mining the fourth international spoligotyping database (SpolDB4) for classification, population genetics and epidemiology. *BMC Microbiol.* 6:23. doi:10.1186/1471-2180-6-23.
- Niobe-Eyangoh SN, Kuaban C, Sorlin P, Cunin P, Thonnon J, Sola C, Rastogi N, Vincent V, Gutierrez MC. 2003. Genetic biodiversity of Mycobacterium tuberculosis complex strains from patients with pulmonary tuberculosis in Cameroon. *J. Clin. Microbiol.* 41:2547–2553.
- Niobe-Eyangoh SN, Kuaban C, Sorlin P, Thonnon J, Vincent V, Gutierrez MC. 2004. Molecular characteristics of strains of the Cameroon family, the major group of Mycobacterium tuberculosis in a country with a high prevalence of tuberculosis. *J. Clin. Microbiol.* 42:5029–5035.
- Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, Bunschoten A, Molhuizen H, Shaw R, Goyal M, van Embden J. 1997. Simultaneous detection and strain differentiation of Mycobacterium tuberculosis for diagnosis and epidemiology. *J. Clin. Microbiol.* 35: 907–914.
- de Jong BC, Antonio M, Awine T, Ogungbemi K, de Jong YP, Gagneux S, DeRiemer K, Zozio T, Rastogi N, Borgdorff M, Hill PC, Adegbola RA. 2009. Use of spoligotyping and large sequence polymorphisms to study the population structure of the Mycobacterium tuberculosis complex in a cohort study of consecutive smear-positive tuberculosis cases in the Gambia. *J. Clin. Microbiol.* 47:994–1001.

8. Brosch R, Gordon SV, Marmiesse M, Brodin P, Buchrieser C, Eiglmeier K, Garnier T, Gutierrez C, Hewinson G, Kremer K, Parsons LM, Pym AS, Samper S, van Soolingen D, Cole ST. 2002. A new evolutionary scenario for the *Mycobacterium tuberculosis* complex. *Proc. Natl. Acad. Sci. U. S. A.* 99:3684–3689.
9. Parwati I, van Crevel R, van Soolingen D. 2010. Possible underlying mechanisms for successful emergence of the *Mycobacterium tuberculosis* Beijing genotype strains. *Lancet Infect. Dis.* 10:103–111.
10. Godreuil S, Torrea G, Terru D, Chevenet F, Diabougou S, Supply P, Van de Perre P, Carriere C, Banuls AL. 2007. First molecular epidemiology study of *Mycobacterium tuberculosis* in Burkina Faso. *J. Clin. Microbiol.* 45:921–927.
11. Gomgnimbou M, Refregier G, Diabougou S, Adama S, Kabore A, Ouiminga A, Sola C. 2012. Spoligotyping of *Mycobacterium africanum*, Burkina Faso. *Emerg. Infect. Dis.* 18:117–119.
12. Thumamo B, Asuquo A, Abia-Bassey L, Lawson L, Hill V, Zozio T, Emenyonu N, Eko F, Rastogi N. 2012. Molecular epidemiology and genetic diversity of *Mycobacterium tuberculosis* complex in the Cross River State, Nigeria. *Infect. Genet. Evol.* 12:671–677.
13. Yeboah-Manu D, Asante-Poku A, Bodmer T, Stucki D, Koram K, Bonsu F, Pluschke G, Gagneux S. 2011. Genotypic diversity and drug susceptibility patterns among *M. tuberculosis* complex isolates from South-Western Ghana. *PLoS One* 6:e21906. doi:10.1371/journal.pone.0021906.
14. Keating L, Wheeler P, Mansoor H, Inwald J, Dale J, Hewinson R, Gordon S. 2005. The pyruvate requirement of some members of the *Mycobacterium tuberculosis* complex is due to an inactive pyruvate kinase: implications for in vivo growth. *Mol. Microbiol.* 56:163–174.
15. Intemann C, Thye T, Niemann S, Browne E, Amanua Chinbuah M, Enimil A, Gyapong J, Osei I, Owusu-Dabo E, Helm S, Rusch-Gerdes S, Horstmann R, Meyer C. 2009. Autophagy gene variant IRGM-261T contributes to protection from tuberculosis caused by *Mycobacterium tuberculosis* but not by *M. africanum* strains. *PLoS Pathog.* 5:e1000577. doi:10.1371/journal.ppat.1000577.
16. Lawson L, Zhang J, Gomgnimbou M, Abdurrahman S, Le Moullec S, Mohamed F, Uzoewulu G, Sogaolu O, Goh K, Emenyonu N, Refregier G, Cuevas L, Sola C. 2012. A molecular epidemiological and genetic diversity study of tuberculosis in Ibadan, Nnewi and Abuja, Nigeria. *PLoS One* 7:e38409. doi:10.1371/journal.pone.0038409.
17. van der Spuy G, Kremer K, Ndabambi S, Beyers N, Dunbar R, Marais B, van Helden P, Warren R. 2009. Changing *Mycobacterium tuberculosis* population highlights clade-specific pathogenic characteristics. *Tuberculosis (Edinb.)* 89:120–125.